Response to the reviewers about the manuscript 34905: **Impact of homogeneous pathologic response to preoperative chemotherapy in patients with multiple colorectal liver metastases**

We thank the reviewers for their comments; all were justified and improved the quality of the study.

Reviewer 1

Overall it's a valuable study with clinically relevant questions. However, there are several major limitations that hamper the definite conclusions in the present form.

1) It's a retrospective study and a large number of factors that are present in personalized everyday patients care are not taken to account

Response: We agree with the reviewer that the retrospective design of the study is a limitation. We have discussed this point in the manuscript. Moreover we have included data on molecular tumor biology.

Modified version, page 10, discussion: One can argue that the retrospective design of the study is a limitation since no special analysis or additional slide for each metastasis was performed.

2) The molecular tumor biology is not taken to account (MSI; BRAF; KRAS; number of therapies etc.)

Response: We thank the reviewer for his comment; we have modified the manuscript to take into account these factors and add these factors in the analysis

Modified version, table 3:

Variable	Homogeneity (Rubbia–Brandt)				Homogeneity (MD – Anderson)			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	OR [95%CI]	P value	OR [95%CI]	P value	OR [95%CI]	P value	OR [95%CI]	P value
Age	2.33 [0.89 – 6.07]	0.82	/	/	1.5 [0.61 – 3.67]	0.37	/	/
Gender	1 [0.48 – 2.09]	0.99	/	/	1 [0.48 – 2.09]	0.99	/	/
Hypertension	1.13 [0.43 – 2.92]	0.81	/	/	1.13 [0.43 – 2.92]	0.81	/	/
Body mass index	0.99 [0.98 – 1.02]	0.95	/	/	1.01 [0.98 – 1.03]	0.86	/	/
Rectal cancer	1.14 [0.56 – 2.34]	0.72	/	/	1.73 [0.82 – 3.63]	0.15	/	/
Number of peroperative LM	0.96 [0.86 – 1.07]	0.45	/	/	0.99 [0.89 – 1.10]	0.87	/	/
Time interval between chemotherapy and surgery	3 [0.31 – 28.84]	0.34	/	/	1.5 [0.53 – 4.21]	0.44	/	/
Folfiri-based chemotherapy	0.007[0.09 – 0,6]	0.9			0.8 [0.3 – 2]	0.6		
Metachronous liver metastases	2.11 [0.96 – 4.67]	0.14	2.8 [0.92 – 8.5]	0.06	1.33 [0.63 – 2.82]	0.45	/	/
T stage	1.26 [0.73 – 2.18]	0.41	/	/	1.17 [0.68 – 2.01]	0.58	/	/
N0 stage	0.8 [0.22 – 2.98]	0.74	/	/	0.8 [0.22 – 2.98]	0.74	/	/
ASA score	1.05 [0.88 – 1.25]	0.62	/	/	1.05 [0.88 – 1.26]	0.56	/	/
MSI	1.9 [0.2 – 18.3]	0.9			1.5 [0.2 – 9.8]	0.6		
RAS status	1.05 [0.0 –99]	0.9			4.5 [0.8- 23.9]	0.3		
Braf mutation	1.6 [0.0 – 120]	0.9			3.3 [0.32 – 34.6]	0.3		
Use of bevacizumab	**3.20 [1.17 – 8.74]**	**0.02**	**3.5 [1.2 – 10.5]**	**0.02**	1.33 [0.56 – 3.16]	0.51	/	/
Metastases in the left lobe of the liver	0.67 [0.24 – 1.87]	0.44	/	/	0.67 [0.24 – 1.87]	0.44	/	/
Number chemotherapy cycles	1.79 [0.93 – 3.44]	0.12	1.06 [0.97 – 1.1]	0.1	1.44 [0.76 – 2.72]	0.27	/	/

3) Location of the primary tumor is not taken into account.

Response: We thank the reviewer for his comment; we have modified the manuscript to take into account these factors and it is included in the analysis of factors associated with homogeniety.

Modified version, page 7, results: The primary tumor was located in the colon in 66% of patients (n=48). It was on the ascending colon in 14% (n=14), on the transverse colon in 7% (n=5), on the descending colon in 45% (n=45) and in the rectum in 34% (n=25). The primary tumour was resected in 98% (n=72) of the cases with a mean delay between the primary tumour resection and the first liver resection of 15.2 months (range: 2-60).

4) The number of the patients is way too small to address those concerns. To my point of view, the study can still address the differences of homogeneous vs. heterogeneous response- but I doulbt that this study would allow a strong conclusion to PRPC and its values in clinical settings. Several points would be valuable to address in revision.

Response: We thank the reviewer for his comment, we agree with his judgement and we have removed survival data of the article.

1) Were the pathologists blinded for the results of the second pathologist?

Response: We thank the reviewer for his comment. The pathologists were blinded from each other.

Modified version page 6: The pathologists were blinded from each other for the analysis but in the event of disagreement between the two pathologists, a consensus was reached.

2) What is the homogeneity/heterogeneity within the single metastasis?

Response: We thank the reviewer for his comment. In a single metastasis, there is variable degree of fibrosis but there is no classification for the degree of homogeneity of the pathological response in a single metastasis since the classifications used are based on the degree of residual cancer cells in a metastasis. We have add this point in the discussion.

Modified version, page 10, discussion: In the present study, a mean of 10 slides per metastasis were prepared, and metastases measuring less than two centimeters were fully embedded [6]. No information on the distribution of the residual tumour cells in a single metastasis is available since there is no classification for that particular point.

3) What are the differences in BRAF/KRAS mutation status? Location of primary tumor?

Response: As requested by the reviewer we have added the Braf and Kras status and the location of the primary tumour.

Modified version, page 7, results: The primary tumor was located in the colon in 66% of patients (n=48). It was on the ascending colon in 14% (n=14), on the transverse colon in 7% (n=5), on the descending colon in 45% (n=45) and in the rectum in 34% (n=25). The primary tumour was resected in 98% (n=72) of the cases with a mean delay between the primary tumour resection and the first liver resection of 15.2 months (range: 2-60). The median number of LM was 3 (range: 1-14), and metastases were synchronous with the primary tumor in 75% of patients (n=55). The rate of patients with BRAF mutation was 5% (n=4). The rate of patients with KRAS mutation was 9.5% (n=7).

4) Were the primary tumors resected, and if yes at what time point?

Response: We thank the reviewer for his comment on this point. We have added this point in the manuscript.

Modified version, page 7, results: The primary tumour was resected in 98% (n=72) of the cases with a mean delay between the primary tumour resection and the first liver resection of 15.2 months (range: 2-60).

5) What is the correlations between RTG and MD Anderson classification? Could the authors present the data more clearly as simple number presentation as in Table 2

Response: As suggested by the reviewers we have indicated more precisely the value of the correlation between the two classifications.

Modified version, page 8: A concordance was observed between the two classifications in 69% of cases (n=61).

6) Why P value.

Response: We thank the reviewer for his comment. As suggested, the relative Small size of the study make conclusions on survival hard to draw. We have removed as suggested data on survival and thus the p value.

Reviewer 2

The authors evaluated the association between pathologic response of preoperative chemotherapy (PRCP) and survival in patients with colorectal liver metastases using major two classifications, and concluded that homogenous PRCP does not associate with overall survival. I have several concerns about this study.

Response: We thank the reviewer for his comment, we have added the information on the presence of other metastases, we have added the criteria for induction of PRPC and added if the primary tumoir was removed or not.

Modified version, page 6, patients and methods: . In the situation of liver metastases, at our institution, all patients had preoperative chemotherapy except for patients with small LM that could disappear with preoperative chemotherapy or patients with a limited number of metastases who had the resection of the primary tumour during the same procedure than liver resection. The decision to use a target agent was considered on a case-by-case basis.

2. Evaluated criteria When did you measure these criteria, particularly tumor size? Is it before or after chemotherapy?

Response: We thank the reviewer for his comment, the tumour size was based on pathological data and were performed after chemotherapy. We have added this point in the manuscript.

Modified version, page 5, patients and method: *Tumor-related:* number of metastases, size of the metastases (after chemotherapy on pathological exam), site in the liver (central or peripheral), primary tumor stage (according to the TNM classification) and site (colon or rectum), and tumor markers (CEA, Ca 19.9).

3. Endpoints Please write the definition of overall survival

Response: We thank the reviewer for his comment. Since some reviewer outline the lack of patients in this study to conclude on survival we have removed survival from the studied criteria.

4. Results

4-1) It is better to change mean follow up to median follow up.

Response: We thank the reviewer for his comment. We have changed the mean follow up to the median follow up.

Modified version, page 7, Results: Median follow-up was 17 months ±15.4.

4-2) This study includes both synchronous and metachronous metastases together, but the survival is worse in metachronous metastases than that of synchronous metastases. Therefore, subgroup analysis is needed although it does not affect to homogeneity in logistic regression model in this study.

Response: We thank the reviewer for his comment, as suggested by one of the reviewer, we have removed data on survival and thus did not do subgroup analysis.

4-3) How did you select the factors to put in logistic regression model? The use of FOLFOX/FOLFIRI should be included. Are there any reason to select the cut-off of 3cm for tumor size and 9 for the number of chemo cycles? Did you put continuous value or dichotomized value in this analysis? The HR of colon cancer and rectal cancer should be opposite but it does not. Please check again.

Response: We thank the reviewer for his comment. We agree that the type of chemotherapy should be included in the logistic regression model. We have added it in the univariate analysis. The use of Folfiri was not significantly associated with homogenous PRPC using the Rubbia-Brandt classification: OR (95%CI)= 0.007 [0.09 – 0,6] or with the MD Anderson (odds ratio (OR) [95% confidence interval (CI)]:0.8 [0.3 – 2]. We have added this in the table 3. We also have removed the cut-off of 3 cm and 9 cycles of chemortherapy that were coming from very first version of our paper with less patiens. In this analysis the number of

chemotherapy cycle was associated with the homogenous PRPC in the Rubbia-Brandt classification and the size of the tumour with the homogenous PRPC in the MD Anderson classification, at that point we have determined these cut-off by a roc curve analysis and keep it in the following version but it does not make sense.

Finally, the only factor that remained significantly different between groups in multivariate analysis for an homogenous PRPC for the Rubbia-Brandt classification was the use of bevacizumab, for the MD Anderson, no factor was associated with a homogeneous PRPC.

Modified version, page 9, results: For the Rubbia-Brandt classification, only the use of bevacizumab (odds ratio (OR) [95% confidence interval (CI)]: 3.5 [1.2- 10.5]; p=0.02) was associated with a homogeneous PRPC **(Table 3)**.

For the MD Anderson classification, no factor was associated with a homogeneous PRPC **(Table 3)**.

Modified version, table 3:

Variable	Homogeneity (Rubbia–Brandt)				Homogeneity (MD – Anderson)			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	OR [95%CI]	P value	OR [95%CI]	P value	OR [95%CI]	P value	OR [95%CI]	P value
Age	2.33 [0.89 – 6.07]	0.82	/	/	1.5 [0.61 – 3.67]	0.37	/	/
Gender	1 [0.48 – 2.09]	0.99	/	/	1 [0.48 – 2.09]	0.99	/	/
Hypertension	1.13 [0.43 – 2.92]	0.81	/	/	1.13 [0.43 – 2.92]	0.81	/	/
Body mass index	0.99 [0.98 – 1.02]	0.95	/	/	1.01 [0.98 – 1.03]	0.86	/	/
Rectal cancer	1.14 [0.56 – 2.34]	0.72	/	/	1.73 [0.82 – 3.63]	0.15	/	/
Number of peroperative LM	0.96 [0.86 – 1.07]	0.45	/	/	0.99 [0.89 – 1.10]	0.87	/	/
Time interval between chemotherapy and surgery	3 [0.31 – 28.84]	0.34	/	/	1.5 [0.53 – 4.21]	0.44	/	/
Folfiri-based chemotherapy	0.007[0.09 – 0,6]	0.9			0.8 [0.3 – 2]	0.6		
Metachronous liver metastases	2.11 [0.96 – 4.67]	0.14	2.8 [0.92 – 8.5]	0.06	1.33 [0.63 – 2.82]	0.45	/	/
T stage	1.26 [0.73 – 2.18]	0.41	/	/	1.17 [0.68 – 2.01]	0.58	/	/
N0 stage	0.8 [0.22 – 2.98]	0.74	/	/	0.8 [0.22 – 2.98]	0.74	/	/
ASA score	1.05 [0.88 – 1.25]	0.62	/	/	1.05 [0.88 – 1.26]	0.56	/	/
MSI	1.9 [0.2 – 18.3]	0.9			1.5 [0.2 – 9.8]	0.6		
RAS status	1.05 [0.0 –99]	0.9			4.5 [0.8- 23.9]	0.3		
Braf mutation	1.6 [0.0 – 120]	0.9			3.3 [0.32 – 34.6]	0.3		
Use of bevacizumab	**3.20 [1.17 – 8.74]**	**0.02**	**3.5 [1.2 – 10.5]**	**0.02**	1.33 [0.56 – 3.16]	0.51	/	/
Metastases in the left lobe of the liver	0.67 [0.24 – 1.87]	0.44	/	/	0.67 [0.24 – 1.87]	0.44	/	/
Number chemotherapy cycles	1.79 [0.93 – 3.44]	0.12	1.06 [0.97 – 1.1]	0.1	1.44 [0.76 – 2.72]	0.27	/	/

4-4) In table 2, how did you calculate the rate of site of metastases? It is strange the sum is 100%. And in preoperative chemotherapy section of table 2, FOLFIRI should be combined with Campto or FOLFIRI with or without cetuximab.

Response: We thank the reviewer for his comment. In table 2, the site of metastases is the site of the 300 metastases in all patients, we have add this point in the table. Moreover, as suggested by the reviewer we combined Campto with folfiri.

Modified version, table 2:

Variable	Study population
Demographic data	
• male gender, n (%)	45 (61)
• Age, median (range), years	62.5 (40 – 80)
• body mass index, mean ± SD, kg/m^2	25.36 ± 4.42
Tumor markers	
• CEA level, mean ± SD (mg/l)	17 ± 3.5
• Ca 19.9 level, mean ± SD (UI/l)	23 ± 5.2
Primary tumor site, n (%):	
• ascending colon	10 (14)
• transverse colon	5 (7)
• descending colon	33 (45)
• rectum	25 (34)
Liver metastases	
• Median (range) number of preoperative LM	3 (1 – 14)
• Synchronous LM, n (%)	55 (75)
• Surgical procedure, n (%)	
o right hepatectomy	15(16)
o left lobectomy	4 (4)
o right lobectomy	3 (3)
o posterior segmentectomy	8 (11)
o wedge	58 (66)
• Two-stage hepatectomy	15 (17)
Site of the 300 metastases (%)	
• I	2.5
• II	10
• III	17.5
• IV	11
• V	16
• VI	20
• VII	13
• VIII	10
Preoperative chemotherapy	
• Regimen, n (%):	
o Folfox	28 (32)
o Folfiri/ Folfox and bevacizumab	28 (32)
o Folfiri with or without cetuximab	8 (9)
o Campto or folfiri with or without cetuximab	20 (23)
o Folfirinox	4 (4)
• Median (range) number of preoperative cycles	12 (4 – 38)
Pathology	
• T Stage, n (%):	
o 2	7 (9)
o 3	56 (77)
o 4	10 (14)
• N Stage, n (%):	
o 0	18 (24)
o 1	37 (51)
o 2	11 (15)
o X	7 (10)
• Median (range) size of metastases, cm	3.1 cm (0.2-5)
PRPC	
• Rubbia-Brandt classification	
o Major response, n (%)	13 (15)
o Partial response, n (%)	12 (14)
o Absence of response, n (%)	63 (71)
• MD Anderson classification	
o Complete response, n (%)	8 (9)
o Major response, n (%)	26 (30)
o Minor response, n (%)	54 (61)

4-5) The number of the metastases may affect the homogeneity, because theoretically the more the site to evaluate, the more the chance of heterogeneity increases. (Besides, the more the site of metastases increases the OS gets worse.)

Response: We thank the reviewer for his comment. We agree with his comment, nevertheless the number of metastases has been evaluated in the multivariate analysis but this was not associated with the homogeneity either with the Rubbia Brandt classification or the MD Anderson classification

4-6) Please show the statistical power to conclude your results.

Response: As underlined by some reviewers, the number of patients is too small to address conclusion on survival. We have thus remove data on survival. Moreover the power for the analysis of the factors associated with a homogeneous PRPC is low since there is a lot of variable studied for a limited number of patients and events. We have discussed this point in the manuscript.

Modified version, page 11, discussion: The outcomes of this analysis should nevertheless interpret with caution, since there was a lot of tested variable of a limited number of patients and events [17].

Reviewer 3

This is a useful paper for me. Preoperative chemotherapy, limited response and not prognostic.

Response: We thank the reviewer for his comment

Reviewer 4

The authors review a cohort of 73 patients undergoing liver resection for colorectal liver metastases after systemic chemotherapy in order to assess the impact of homogeneity of pathological response to chemotherapy on survival and routine management of patients. The pathological response was homogeneous in only one half of patients. Heterogeneity of pathological response did not influence overall survival The authors conclude that pathological response to chemotherapy is not a powerful prognostic factor and do not influence treatment or management in patients with advanced resectable liver metastases. Overall this is a concise and well written manuscript. However a number of issues must be addressed.

1) The definition of heterogeneity of pathological response is not clear and should be clarified. In particular, what is the definition of heterogeneity of pathological response according different histological classifications?

Response: We thank the reviewer for his comment. We have clarified the definition of the homogeneity, heterogeneity.

Modified version, page 5, patients and methods: a homogeneous PRPC (defined as the same classification for all metastases resected in a given patient, for example all metastases were classified as having a major regression when Rubbia-Brandt classification is considered and for example all metastases had a minor response to PRPC when the MD Anderson classification is considered). A heterogenous PRPC was determined if in a single specimen, one metastasis had a major regression and others have partial regression or no regression in the Rubbia-Brandt classification or if in a single specimen, one metastasis had a major PRPC and others have complete or minor PRPC in the MD Anderson classification.

2) The authors claim that that the pathological response did not influence survival and MDT decision in routine practice. The data presented did not provide adequate evidence

to support this statement. No conclusion can be made from a single centre study about the impact of pathological response on management of patients. The authors did not take into account pathological response but it could be different in another centre. Furthermore the authors have shown that heterogeneity of pathological response had no impact on survival but they can conclude that pathological response is not a prognosis factor. In other word heterogeneous response does not mean absence of response. The authors can made only conclusion about the impact of heterogeneity of pathological response.

Response: We agree with the reviewer, this is one of the main criticism of the reviewers. We have removed all data on survival

3) What is the impact of heterogeneity of pathological response on disease free survival ?

Response: As asked by several reviewers, we have removed data on survival

4) The impact of heterogeneity on survival and disease free survival should be analysed in the entire cohort using univariate logistic regression analysis.

Response: As asked by several reviewers, we have removed data on survival

5) Abbreviation MDT should be defined

Response: We thank the reviewer for his comment, we have defined the abbreviation MDT.

Modified version, page 4, introduction: The objective of the present study was therefore to analyze the homogeneity of PRPC after chemotherapy and to assess the impact of PRPC on the multidisciplinary team meeting (MDT) decision